## Claims

1. A method for labeling a target protein comprising

contacting a fusion protein with a biotin analog, and

allowing sufficient time for the biotin analog to be conjugated to the fusion protein via an acceptor peptide, in the presence of a biotin ligase mutant,

wherein the fusion protein is a fusion of the target protein and the acceptor peptide.

2. The method of claim 1, wherein the biotin analog comprises an aliphatic carboxylic acid tail.

3. The method of claim 1, wherein the biotin analog comprises a substitution at a trans-ureido nitrogen (N) of biotin.

4. The method of claim 1, wherein the biotin analog is selected from the group consisting of an N-ketone biotin analog, a ketone biotin analog, an N-azide biotin analog, an azide biotin analog, an N-acyl azide biotin analog, an NBD-GABA biotin analog, a 1,2-diamine biotin analog, an N-alkyne biotin analog and a tetrathiol biotin analog.

5. The method of claim 1, wherein the biotin analog is fluorogenic.

6. The method of claim 1, wherein the biotin analog is directly detectable.

7. The method of claim 6, wherein the biotin analog is coumarin, fluorescein, rhodamine, rosamine, an Alexa™ dye, resorufin, oregon green, tetramethyl rhodamine, Texas Red® or BODIPY.

8. The method of claim 1, wherein the biotin analog is labeled with a directly detectable label.

9. The method of claim 8, wherein the directly detectable label is selected from the group consisting of a fluorophore, a radioisotope, a contrast agent, an MRI contrast agent, a PET label, a phosphorescent label and a luminescent label.

10. The method of claim 1, wherein the biotin analog is labeled with an indirectly detectable label.

11. The method of claim 10, wherein the indirectly detectable label is selected from the group consisting of an enzyme, an enzyme substrate, an antibody, an antibody fragment, an antigen, a hapten, a ligand, an affinity molecule, a chromogenic substrate, a protein, a peptide, a nucleic acid, a carbohydrate and a lipid.

12. The method of claim 1, wherein the biotin analog is labeled with a membrane impermeant label.

13. The method of claim 1, wherein the biotin analog is labeled after conjugation to the fusion protein.

14. The method of claim 1, wherein the biotin analog is labeled with a singlet oxygen radical generator.

15. The method of claim 14, wherein the singlet oxygen generator is resorufin, malachite green, fluorescein or diaminobenzidine.

16. The method of claim 1, wherein the biotin analog is labeled with an analyte-binding group.

17. The method of claim 16, wherein the analyte-binding group is a metal chelator.

18. The method of claim 17, wherein the metal chelator is EDTA, EGTA, a pyridinium, an imidazole or a thiol.

19. The method of claim 1, wherein the biotin analog is labeled with a heavy atom carrier.

20. The method of claim 19, wherein the heavy atom carrier is iodine.

21. The method of claim 1, wherein the biotin analog is labeled with an affinity tag.

22. The method of claim 21, wherein the affinity tag is selected from the group consisting of a histidine tag, a GST tag, a FLAG tag and an HA tag.

23. The method of claim 1, wherein the biotin analog is labeled with a photoactivatable cross-linker.

24. The method of claim 23, wherein the photoactivatable cross-linker is selected from the group consisting of benzophenones and aziridines.

25. The method of claim 1, wherein the biotin analog is labeled with a photoswitch label.

26. The method of claim 25, wherein the photoswitch label is an azobenzene.

27. The method of claim 1, wherein the biotin analog is labeled with a photolabile protecting group.

28. The method of claim 27, wherein the photolabile protecting group is a nitrobenzyl group, a dimethoxy nitrobenzyl group or NVOC.

29. The method of claim 1, wherein the biotin analog is labeled with a peptide comprising non-naturally occurring amino acids.

30. The method of claim 1, wherein the target protein is a cell surface protein.

31. The method of claim 1, wherein the fusion protein is in a cell.

32. The method of claim 31, wherein the cell expresses the biotin ligase mutant.

33. The method of claim 31, wherein the cell is a eukaryotic cell.

34. The method of claim 31, wherein the cell is a bacterial cell.

35. The method of claim 33, wherein the eukaryotic cell is a mammalian cell, a Drosophila cell, a Zebrafish cell, a Xenopus cell, a yeast cell or a C. elegans cell.

36. The method of claim 1, wherein the acceptor peptide comprises an amino acid sequence of SEQ ID NO: 4.

37. The method of claim 1, wherein the acceptor peptide comprises an amino acid sequence of SEQ ID NO: 5.

38. The method of claim 1, wherein the acceptor peptide is N- or C- terminally fused to the target protein.

39. The method of claim 1, wherein the biotin ligase mutant has an amino acid substitution at 83, 89, 90, 91, 92, 107, 112, 115, 116, 117, 118, 123, 132, 134, 142, 186, 188, 189, 190, 204, 206, 207 and/or 235.

40. The method of claim 39, wherein the amino acid substitution is at T90, C107, Q112, G115, Y132, S134, V189 and/or I207.

41. The method of claim 40, wherein the amino acid substitution is at T90.

42. The method of claim 41, wherein the amino acid substitution is selected from the group consisting of T90G, T90A and T90V.

43. The method of claim 42, wherein the amino acid substitution is T90G.

44. The method of claim 43, wherein the biotin analog is N-ketone biotin analog.

45. The method of claim 43, wherein the biotin ligase mutant has an amino acid sequence of SEQ ID NO: 6.

46. The method of claim 41, wherein the biotin ligase mutant further comprises an amino acid substitution at N91.

47. The method of claim 46, wherein the amino acid substitution at N91 is N91S, N91G, N91A or N91L.

48. The method of claim 47, wherein the biotin ligase mutant comprises amino acid substitutions of T90G and N91S.

49. The method of claim 48, wherein the biotin analog is N-alkyne biotin analog.

50. The method of claim 48, wherein the biotin ligase mutant has an amino acid sequence of SEQ ID NO: 7.

51. The method of claim 1, wherein the biotin ligase mutant comprises amino acid substitutions of T90G/N91G, T90A/N91A or T90A/N91L.

52. The method of claim 39, wherein the amino acid substitution is C107G, Q112M, G115A, Y132G, Y132A, S134G, V189G and/or I207S.

53. The method of claim 1, wherein the method is performed in a cell free environment.

54. The method of claim 1, wherein the method is performed in a cell.

55. The method of claim 1, wherein the method is performed in a subject.

56. The method of claim 1, wherein the acceptor peptide is fused to the target protein via a cleavable bond or linker.

57. A composition comprising
a biotin ligase mutant that binds to a biotin analog.

58. The composition of claim 57, wherein the biotin ligase mutant comprises an amino acid substitution in a biotin interaction and activation domain.

59. The composition of claim 57, wherein the biotin ligase mutant comprises an amino acid substitution at 83, 89, 90, 91, 92, 107, 112, 115, 116, 117, 118, 123, 132, 134, 142, 186, 188, 189, 190, 204, 206, 207 and/or 235.

60. The composition of claim 59, wherein the amino acid substitution is at T90, C107, Q112, G115, Y132, S134, V189 and/or I207.

61. The composition of claim 60, wherein the amino acid substitution is at T90.

62. The composition of claim 61, wherein the amino acid substitution is selected from the group consisting of T90G, T90A and T90V.

63. The composition of claim 62, wherein the amino acid substitution is T90G.

64. The composition of claim 63, wherein the biotin ligase mutant has an amino acid sequence of SEQ ID NO: 6.

65. The composition of claim 61, wherein the biotin ligase mutant further comprises an amino acid substitution at N91.

66. The composition of claim 65, wherein the amino acid substitution at N91 is N91S, N91G, N91A or N91L.

67. The composition of claim 66, wherein the biotin ligase mutant comprises amino acid substitutions of T90G and N91S.

68. The composition of claim 67, wherein the biotin ligase mutant has an amino acid sequence of SEQ ID NO: 7.

69. The composition of claim 59, wherein the amino acid substitution is C107G, Q112M, Q112G, G115A, Y132G, Y132A, S134G, V189G and/or I207S.

70. The composition of claim 57, wherein the biotin ligase mutant is isolated.

71. The composition of claim 57, wherein the biotin ligase mutant has reduced binding affinity to biotin.

72. The composition of claim 57, wherein the biotin ligase mutant has wild type binding affinity to biotin.

73. The composition of claim 57, wherein the biotin analog comprises a substitution at a trans-ureido nitrogen (N) of biotin.

74. The composition of claim 73, wherein the biotin analog is an N-ketone biotin analog or an N-alkyne biotin analog.

75. The composition of claim 57, wherein the biotin analog is an azide biotin analog, an N-acyl azide biotin analog or an N-azide biotin analog.

76. The composition of claim 57, wherein the biotin analog is an NBD-GABA biotin analog or a 1,2-diamine biotin analog.

77. The composition of claim 57, wherein the biotin analog is a tetrathiol biotin analog.

78. The composition of claim 57, wherein the biotin analog is a ketone biotin analog.

79. The composition of claim 57, wherein the biotin analog is coumarin, fluorescein, rhodamine, rosamine, an Alexa™ dye, resorufin, oregon green, Texas Red®, tetramethyl rhodamine or BODIPY.

80. The composition of claim 57, wherein the biotin analog is fluorogenic.

81. A composition comprising

a nucleic acid encoding a biotin ligase mutant comprising an amino acid substitution at 83, 89, 90, 91, 92, 107, 112, 115, 116, 117, 118, 123, 132, 134, 142, 186, 188, 189, 190, 204, 206, 207 and/or 235.

82. The composition of claim 81, wherein the amino acid substitution is selected from the group consisting of T90G, T90A, T90V, N91S, N91G, N91A, N91L, C107G, Q112M, Q112G, G115A, Y132G, Y132A, S134G, V189G, and I207S.

83. The composition of claim 82, wherein the amino acid substitution is selected from the group consisting of T90G, T90A and T90V.

84. The composition of claim 83, wherein the amino acid substitution is T90G.

85. The composition of claim 84, wherein the nucleic acid has a sequence of SEQ ID NO: 6.

86. The composition of claim 83, wherein the biotin ligase mutant further comprises an amino acid substitution at N91.

87. The composition of claim 86, wherein the amino acid substitution at N91 is N91S, N91G, N91A or N91L.

88. The composition of claim 87, wherein the amino acid substitution at N91 is N91S.

89. The composition of claim 88, wherein the nucleic acid has a sequence of SEQ ID NO: 7.

90. The composition of claim 81, wherein the nucleic acid is isolated.

91. A vector comprising the nucleic acid of claim 81-89 or 90.

92. A host cell comprising the vector of claim 91.

93. The host cell of claim 92, wherein the nucleic acid is inducibly expressed.

94. A process for preparing a biotin ligase mutant comprising culturing the host cell of claim 92 or 93 and recovering the biotin ligase mutant from the culture.

95. A composition comprising
a biotin analog that binds to a biotin ligase mutant,
wherein the biotin analog is alkylated at a trans-ureido nitrogen (N) of biotin.

96. The composition of claim 95, wherein the biotin analog is selected from the group consisting of an N-ketone biotin analog, an N-azide biotin analog, an N-acyl azide biotin analog, and an N-alkyne biotin analog.

97. The composition of claim 95, wherein the biotin analog is not recognized by wild type biotin ligase.

98. The composition of claim 95, wherein the biotin ligase mutant comprises an amino acid substitution at 83, 89, 90, 91, 92, 107, 112, 115, 116, 117, 118, 123, 132, 134, 142, 186, 188, 189, 190, 204, 206, 207 or 235.

99. The composition of claim 98, wherein the amino acid substitution is at T90, G115, Y132, C107, Q112, V189, I207 or S134.

100. The composition of claim 99, wherein the amino acid substitution is at T90.

101. The composition of claim 100, wherein the amino acid substitution is selected from the group consisting of T90G, T90A and T90V.

102. The composition of claim 101, wherein the amino acid substitution is T90G.

103. The composition of claim 102, wherein the biotin ligase mutant has an amino acid sequence of SEQ ID NO: 6.

104. The composition of claim 100, wherein the biotin ligase mutant further comprises an amino acid substitution at N91.

105. The composition of claim 104, wherein the amino acid substitution at N91 is N91S, N91G, N91A or N91L.

106. The composition of claim 105, wherein the biotin ligase mutant comprises amino acid substitution of T90G and N91S.

107. The composition of claim 106, wherein the biotin ligase mutant has an amino acid sequence of SEQ ID NO: 7.

108. The composition of claim 98, wherein the amino acid substitution is C107G, Q112M, G115A, Y132G, Y132A, V189G, S134G or I207S.

109. The composition of claim 95, wherein the biotin analog is isolated.

110. A composition comprising
a biotin analog that binds to a biotin ligase mutant,
wherein the biotin analog is ketone biotin analog or NBD-GABA.

111. A phage display library comprising a biotin ligase mutant having an amino acid substitution at 83, 89, 90, 91, 92, 107, 112, 115, 116, 117, 118, 123, 132, 134, 142, 186, 188, 189, 190, 204, 206, 207 or 235.

112. The phage display library of claim 111, wherein the amino acid substitution is at T90, G115, Y132 C107, Q112, V189, I207 or S134.

113. The phage display library of claim 112, wherein the amino acid substitution is at T90.

114. The phage display library of claim 113, wherein the amino acid substitution is selected from the group consisting of T90G, T90A and T90V.

115. The phage display library of claim 114, wherein the amino acid substitution is T90G.

116. The phage display library of claim 114, wherein the biotin ligase mutant further comprises an amino acid substitution at N91.

117. The phage display library of claim 116, wherein the amino acid substitution at N91 is N91S, N91G, N91A or N91L.

118. The phage display library of claim 117, wherein the biotin ligase mutant comprises amino acid substitutions of T90G and N91S.

119. The phage display library of claim 111, wherein the amino acid substitution is C107G, Q112M, G115A, Y132G, Y132A, V189G, S134G or I207S.

120. The phage display library of claim 111, wherein the amino acid substitution is at 90, 91, 112, 115, 116, 132 or 188.

121. The phage display library of claim 111, wherein the library has at least about $1 \times 10^9$ members.

122. A method for identifying a biotin ligase mutant having specificity for a biotin analog comprising

contacting a biotin analog with an acceptor peptide in the presence of a candidate biotin ligase mutant molecule, and

detecting a biotin analog that is bound to the acceptor peptide,

wherein the presence of a biotin analog bound to an acceptor peptide indicates that the candidate biotin ligase mutant molecule is a biotin ligase mutant having specificity for a biotin analog.

123. The method of claim 122, wherein the candidate molecule is a library member.

124. The method of claim 123, wherein the library member is a phage display library member.

125. The method of claim 122, wherein the candidate molecule is bound to a solid support.

126. The method of claim 122, wherein the candidate molecule is soluble.

127. The method of claim 122, wherein the biotin analog is directly detectable.

128. The method of claim 122, wherein the biotin analog is selected from the group consisting of coumarin, fluorescein, rhodamine, rosamine, an Alexa™ dye, resorufin, oregon green, tetramethyl rhodamine, Texas Red® and BODIPY.

129. The method of claim 122, wherein the biotin analog is conjugated to a detectable label.

130. The method of claim 129, wherein the detectable label is a directly detectable label.

131. The method of claim 130, wherein the directly detectable label is selected from the group consisting of a fluorophore, a radioisotope, a contrast agent, an MRI contrast agent, a PET label, a phosphorescent label and a luminescent label.

132. The method of claim 129, wherein the detectable label is an indirectly detectable label.

133. The method of claim 132, wherein the indirectly detectable label is selected from the group consisting of an enzyme, an enzyme substrate, an antibody, an antibody fragment, an antigen, a hapten, a ligand, an affinity molecule, a chromogenic substrate, a protein, a peptide, a nucleic acid, a carbohydrate and a lipid.

134. The method of claim 122, wherein the biotin analog is fluorogenic.

135. The method of claim 129, wherein detecting a biotin analog comprises detecting the detectable label conjugated to the biotin analog.

136. The method of claim 122, wherein the acceptor peptide has an amino acid sequence comprising SEQ ID NO: 4.

137. The method of claim 122, wherein the acceptor peptide has an amino acid sequence comprising SEQ ID NO: 5.

138. The method of claim 122, wherein the biotin analog is selected from the group consisting of a ketone biotin analog, an N-ketone biotin analog, an N-alkyl biotin analog, an azide biotin analog, an N-acyl azide biotin analog, an N-azide biotin analog and a tetrathiol biotin analog.

139. The method of claim 122, wherein the biotin analog is NBD-GABA or 1,2-diamine biotin analog.

140. The method of claim 122, wherein the biotin analog is detected using an antibody.

141. The method of claim 122, wherein the biotin analog is detected using a detection system selected from the group consisting of a fluorescent detection system, a luminescent detection system, a photographic film detection system, an enzyme detection system, an electron spin resonance detection system, a scanning tunneling microscopy (STM) detection system, an optical detection system and a nuclear magnetic resonance (NMR) detection system.

142. The method of claim 122, further comprising removing unbound biotin analog prior to detecting bound biotin analog.

143. The method of claim 122, further comprising isolating the candidate molecule that is a biotin ligase mutant having specificity for a biotin analog.

144. The method of claim 122, further comprising identifying a biotin ligase mutant having specificity for a biotin analog and biotin.

145. The method of claim 144, wherein the biotin ligase mutant having specificity for a biotin analog and biotin is identified by

contacting biotin with an acceptor peptide in the presence of a candidate molecule, and

detecting biotin that is bound to the acceptor peptide,

wherein the presence of biotin bound to an acceptor peptide indicates that the candidate molecule is a biotin ligase mutant having specificity for a biotin analog and biotin.

146. A method for identifying a biotin analog having specificity for a biotin ligase mutant comprising

combining an acceptor peptide with a labeled biotin in the presence of a biotin ligase mutant and determining a control level of biotin incorporation,

combining an acceptor peptide with a labeled biotin and a candidate biotin analog molecule in the presence of a biotin ligase mutant and determining a test level of biotin incorporation, and

comparing the control and test levels of biotin incorporation,

wherein a test level that is less than a control level is indicative of a biotin analog having specificity for a biotin ligase mutant.

147. The method of claim 146, wherein the biotin analog comprises an aliphatic carboxylic acid tail.

148. The method of claim 146, wherein the biotin analog comprises a substitution at a trans-ureido nitrogen (N) of biotin.

149. The method of claim 146, wherein the acceptor peptide comprises an amino acid sequence of SEQ ID NO: 4.

150. The method of claim 146, wherein the acceptor peptide comprises an amino acid sequence of SEQ ID NO: 5.

151. The method of claim 146, wherein the biotin ligase mutant comprises an amino acid substitution at 83, 89, 90, 91, 92, 107, 112, 115, 116, 117, 118, 123, 132, 134, 142, 186, 188, 189, 190, 204, 206, 207 or 235.

152. The method of claim 151, wherein the amino acid substitution is at T90, C107, Q112, G115, Y132, S134, V189 or I207.

153. The method of claim 152, wherein the amino acid substitution is at T90.

154. The method of claim 153, wherein the amino acid substitution is selected from the group consisting of T90G, T90A and T90V.

155. The method of claim 154, wherein the amino acid substitution is T90G.

156. The method of claim 155, wherein the biotin ligase mutant has an amino acid sequence of SEQ ID NO: 6.

157. The method of claim 153, wherein the biotin ligase mutant further comprises an amino acid substitution at N91.

158. The method of claim 157, wherein the amino acid substitution at N91 is N91S, N91G, N91A or N91L.

159. The method of claim 158, wherein the biotin ligase mutant comprises amino acid substitutions at T90G and N91S.

160. The method of claim 159, wherein the biotin ligase mutant has an amino acid sequence of SEQ ID NO: 7.

161. The method of claim 151, wherein the amino acid substitution is C107G, Q112M, G115A, Y132G, Y132A, S134G, V189G or I207S.